

## **Western Blotting:**

**Reagent: NAP<sup>TM</sup>-blocker; GenoTech cat#786-190**

### **Procedure:**

- a. **Block non-specific binding on membrane with blocking buffer (NAP<sup>TM</sup>-blocker: TBS with 0.1% Tween 20 = 1:3) for 1 hour at RT**  
**For Smad: Block unspecific binding on membrane with 5% dry milk (Santa Cruz Biotech, cat#sc-2325) in TBS with 0.1% Tween 20 for 1 hour at RT.**  
**Or: Block unspecific binding on membrane with 5% BSA and 1% goat serum in TBS with 0.1% Tween 20 for 20 minutes at RT.**
- b. **Wash membrane 3 times in TBS Tween for 5 min each.**
- c. **Dilute primary antibody with 3% BSA+ 1% serum in TBS with 0.1% Tween 20.**

**Add 5ml for 1 membrane or 10ml for 2-3 membranes above solution into Petri dishes. Add appropriate primary antibodies into dishes and mix well. Add membranes into the appropriate dishes, and incubate for 1 hour at RT.**

**For Smad: Dilute primary antibody with 5% BSA+ 1% serum in TBS with 0.1% Tween 20. Incubate overnight.**

- d. **Wash membrane 3 times in TBS Tween for 5 min each.**
- e. **Add second antibody diluted 40000-fold with blocking buffer incubate for 1 hour at room temperature.**  
**For Smad: dilute second antibody with 5% dry milk in TBS with Tween.**

**Add 20ml to each container (use old pipette tip box top).**

- f. **Wash membrane 3 times in TBS Tween for 5 min each.**
- g. **Mix PIERCE "SuperSignal chemoluminescent Substrate Luminol/Enhancer" with equal amount of PIERCE "SuperSignal chemoluminescent Substrate Stable Peroxide Solution". Add 10ml substrate to container/pouch, stain for 5 min.**
- h. **In Darkroom, expose film to membrane for varying times**
- i. **Develop films.**